REMARKS

Reconsideration and allowance are requested in view of the above amendments to the claims and the following discussion.

Background

The present invention is directed to an equine feed product which is formulated specifically for horses which undergo intensive training in preparation for races. There are problems associated with currently used equine feed products, such as barley and maize. As known in the industry, oats contain less fermentable starch and more fiber than either barley or maize. However, a diet consisting solely of oats is deficient in essential nutrients to varying degrees. A diet with deficiencies of essential nutrients has an adverse effect on a horse's performance.

Consequently, applicant has developed an equine feed product comprising oats and an oat-balancing feed supplement as defined in the claims of this application.

I. The Objection to Claims 4-15

The Examiner has objected to Claims 4-15 under 37 C.F.R. 1.75(c) as being in improper form because these multiple dependent claims are dependent upon other multiple dependent claims.

In response, Claims 4-15 are amended to be in proper dependent form.

II. The Rejections Under Section 102(b)

In 4 separate rejections, the Examiner has rejected Claim 1 under 35 U.S.C. 102(b) as being anticipated by the following 4 references taken separately:

(1) L.A. Lawrence, "Nutrient Requirements and Balancing Rations for Horses"; Virginia Cooperative Extension, Animal and Poultry Sciences; Publication 406-473; 16 pages (2000);

(2) Allen et al. U.S. Patent No. 6,338,856;

(3) Anderson U.S. Patent No. 6,451,370; and

(4) "Nutrient Requirements of Horses"; The National Academy of Sciences; pages 15-17, 23-24, 34, 43-48, 95 (1989).

Applicant notes that Claims 2-3 are not subject to this rejection.

The language of 35 U.S.C. 102(b) states that:

> A person shall be entitled to a patent unless---
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States, or....

The interpretation of 102(b) is, without question, that **the denial of a patent requires that the reference teach applicant's invention as defined by the claims**. This requirement is also referred to as "anticipation", and the Courts have provided clear and unambiguous definitions in this area.

In *General Electric Company v. United States*, 572 F.2d 745, 768, 198 U.S.P.Q. 65, 85 (U.S. Court of Claims 1978), a case involving Section 102(e), the Court states:

> **To anticipate a claim a prior reference must show each and every element claimed**. Short of this, anticipation does not exist. *In re Royka*, 490 F.2d 981, 984, 180 U.S.P.Q. 580, 583 (cust. & Pat. App. 1974).
>
> (Emphasis added.)

Applicant refers to *In re Spada and Wilczynski*, 911 F.2d 705, 15 USPQ2d 1655 (Fed. Cir. 1990)), where the Court states on page 708:

> Rejection for anticipation or lack of novelty requires, as the first step in the inquiry, that **all the elements of the claimed invention** be described in a single reference.... Further, the reference must describe the applicant's claimed invention **sufficiently** to have placed a person of ordinary skill in the field of the invention **in possession of it**...
>
> (Emphasis added and citations omitted.)

Applicant also refers to *Helifix Limited v. Blok-Lok, Ltd.*, 208 F.3d 1339, 54 USPQ 2d 1299 (Fed. Cir. 2000) and *In re Donohue*, 766 F.2d 531, 226 USPQ 619 (Fed. Cir. 1985).

As noted above, Claims 2 - 3 are not subject to this rejection under Section 102(b). Therefore, to clarify the differences between the present invention as defined by applicant's claims and the teachings of the references cited by the Examiner, Claim 1 is amended to incorporate the subject matter of Claims 2 and 3. With these amendments, Claims 2 and 3 are cancelled.

Consequently, applicant requests the removal of this rejection under Section 102(b).

III. The Rejection Under Section 103(a)

The Examiner has rejected Claims 1-15 under 35 U.S.C. 103(a) as being unpatentable over the following primary references:

(a) Allen et al. U.S. Patent No. 6,338,856;

(b) Anderson U.S. Patent No. 6,451,370;

(c) "Nutrient Requirements of Horses"; The National Academy of Sciences; pages 15-17, 23-24, 34, 43-48, 95 (1989);

(d) Australian Patent No. 753033;

(e) Betz et al. U.S. Patent No. 4,166,867;

(f) Russian Patent Publication No. 1391570; and

(g) Ott et al.; "Influence of Protein Level and Quality on the Growth and Development of Yearling Foals"; Journal of Animal Science; Vol. 49, No. 3; pages 620-628 (1979);

in view of the following secondary reference:

(h) Graham et al.; "The Effect of Supplemental Lysine and Threonine on Growth and Development of Yearling Horses"; Journal of Animal Science; Vol 72; pages 380-386 (1994);

and further in view of the following tertiary references:

(i) L.A. Lawrence; "Nutrient Requirements and Balancing Rations for Horses"; Virginia Cooperative Extension, Animal and Poultry Sciences; Publication No. 406-473; 16 pages (2000); and

(j) "Horse Science: Balancing Rations for Horses"; 4-H Horse Program; Unit 2; National 4-H Council, MD 20815; 8 pages (1989).

This rejection is traversed in view of the following reasons.

Section 103(a) requires that, if a patent is denied to an applicant, the differences between the subject matter sought to be patented and the prior art must be such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which the subject matter pertains. Section 103(a) further provides that patentability shall not be negatived by the manner in which the invention was made.

With regard to the requirements for a proper obviousness rejection under Section 103, applicant refers to the following decisions.

The Court of Appeals for the Federal Circuit states as follows in *In re Wright*, 6 U.S.P.Q.2d 1959, 1961 (CAFC 1988):

> We repeat the mandate of 35 U.S.C. § 103: it is the invention as a whole that must be considered in obviousness determinations. **The invention as a whole embraces the structure, its properties, and the problem it solves.**
>
> . . . The determination of whether a novel structure is or is not "obvious" **requires cognizance of the properties of that structure and the problem which it solves**, viewed in light of the teachings of the prior art.
>
> . . . (the particular problem facing the inventor **must be considered** in determining obviousness). . ..
>
> . . . (it is error to focus "solely on the product created, rather than on the obviousness or nonobviousness of its creation"). . ..
>
> (Emphasis added and citations omitted.)

In dealing with the concept of obviousness, the CAFC in the *Wright* case clearly states on pages 1961-2:

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> Thus the question is whether what the inventor did would have been obvious to one of ordinary skill in the art **attempting to solve the problem upon which the inventor was working**.
>
> The problem solved by the invention is always relevant. The entirety of a claimed invention, including the combination viewed as a whole, the elements thereof, and the properties and purpose of the invention, must be considered.
>
> In either case, the requisite view of the whole invention mandates consideration of not only its structure but also its properties and **the problem solved**.
>
> (Emphasis added and citations omitted.)

Applicant maintains that, without knowledge or recognition of his problem (i.e., the objective of applicant's invention), a patent cannot properly be asserted under the concept of obviousness. In regard to applicant's problem, as stated in Paragraph 0008 of the published U.S. application:

> Accordingly, the present invention provides an equine oat-balancing feed supplement comprising a mix of components including lysine, iodine, copper, magnesium, zinc and calcium, where the quantity of lysine present in the oat-balancing feed supplement fed to the animal per day ranges between 3.00 g and 18.00 g according to the age of the animal.

In seeking an equine oat-balancing feed supplement which avoids the problems associated with prior feed regimes, applicant discovered the supplement as defined by the amended claims of this application. This supplement is a new, unique and useful

combination of components. While applicant does not claim that the individual components are new, applicant does claim that the combination is new, unique, useful and not obvious from the prior art cited by the Examiner in the Office Action.

There must be at least a suggestion of applicant's problem for one having ordinary skill in this art to use the ten (10) cited references as a basis or starting point toward a solution to such problem.

This theory is not new, as shown by the Court of Customs and Patent Appeals in *In re Shaffer*, 108 U.S.P.Q.326, 329 (CCPA 1956):

> In fact, a person having the references before him **who was not cognizant** of appellant's disclosure would not be informed that the problem solved by appellant ever existed. **Therefore, can it be said that these references which never recognized appellant's problem would have suggested its solution. We think not**, and therefore feel that the references were improperly combined since there is no suggestion in either of the references that they can be combined to produce appellant's result.
>
> (Emphasis added.)

Specifically, the combination of the seven (7) primary references with the secondary reference and the two (2) tertiary references cited by the Examiner does not provide even a remote recognition of the problem faced by applicant. Therefore, the cited combination of references fails to suggest a solution to applicant's problem.

The Ten (10) Cited References

Reference No. 1

The Allen patent (a primary reference) is directed to a seaweed supplement for the diet of mammals and poultry to enhance immune response. Specifically, as disclosed at lines 13-26 of column 1, this patent discloses:

> The invention relates to seaweed and treated seaweed feed supplements for mammal and poultry wherein the host exhibits enhanced immune response. In another aspect, the invention relates to the introduction of seaweed supplement directly to mammal and poultry feed as well as introduction of grazing animals to plants and grasses, which have been pre-treated with seaweed extract. In still another aspect, the invention relates to seaweed feed supplement, which enhances the host immune system for periods beyond cessation of seaweed supplement introduction to the host diet. Aspects of the invention also relate to imparting resistance to Porcine Reproductive and Respiratory Syndrome (PRRS) disease in pigs that have been exposed to PRRS disease and to mitigating the stress of weaning in lactating mares.

Applicant acknowledges that Example VII of the Allen patent discloses addition of a seaweed extract to a diet for mares and their foals. However, neither this diet nor the remainder of the patent teaches or suggests applicant's feed supplement **with the components in the amounts defined in the amended claims of this application**.

Attached as Exhibit A is a copy of a Conway et al. paper entitled "Equine Goitre"; Irish Veterinary Journal, Vol. 34, No. 3; pages 29-31 (1980). The authors reveal the effects of feeding a substance having a high iodine content to dams during pregnancy and the condition of their foals. The horses were fed oats, bran and a supplementary conditioner which contained 20% seaweed. After birth, the foals showed enlarged thyroid glands and were backward in condition. Consequently, one having ordinary skill in this art would not consider feeding a seaweed supplement to a horse. Therefore, the Allen patent teaches away from the present invention.

Reference No. 2

The Anderson patent (another primary reference) is directed to the use of a potato component which acts as a binder in animal feed. Specifically, at lines 7-19 of column 1, this patent discloses:

> This invention relates generally to food for animals. More specifically, this invention relates to a complete, integral animal feed, especially for horses, cattle, dogs, cats and other domesticated animals. The complete animal feed includes a potato component which acts as a binder to bind all of the components of the feed into an integral, pelletizable product. The potato component is particularly useful for binding a nutritional supplement with the other components. The nutritional supplement will vary depending upon the animal in question. All of the components, including the bound nutritional supplement, are combined and palletized. The resulting pellets

> provide an integral animal feed which alone meets the complete nutritional requirements of the particular animal.

However, as taught by Buckley et al. in "Analysis of Canadian and Irish Forage, Oats and Commercially Available Equine Concentrate Feed for Pathogenic Fungi and Mycotoxins"; Irish Veterinary Journal; Vol. 60, No. 4; pages 231-236 (2007), pelleted feed is more likely to contain T2 toxins than are oats. These toxins interfere with protein synthesis and affect immune response by suppressing antibody formation. (A copy of this document is attached as Exhibit B.)

Therefore, the Anderson patent **fails to teach or suggest the feed supplement of this application** to one having ordinary skill in this art.

Reference No. 3

The "Nutrient Requirements of Horses" reference (a further primary reference) provides commentary on several nutrients which form part of the nutrient requirements for a horse. However, this reference does not teach or suggest a feed for equines, in which the feed comprises oats and the oat-balancing feed supplement **which comprises the components in the amounts as defined in applicant's amended claims**.

Reference No. 4

The Australian patent (a further primary reference) is directed to a low cereal grain feedstuff for a racehorse. Specifically, on page 4, this patent discloses:

> According to one aspect, the invention resides in a method of improving the race performance of a racehorse of a type conventionally fed a feedstuff including a substantial portion of cereal grain wherein at least 36% of the cereal grain component of said feedstuff is replaced by a cereal grain composition comprising cereal grain from which a substantial proportion of endosperm has been removed.

The Australian patent further discloses on page 4:

> Preferably the cereal grain composition is from one of wheat, rice, maize, barley, sorghum, oats, millet, triticale or rye or any combination thereof. **It maybe preferred to exclude oats as a source of cereal grain composition**.
>
> (Emphasis added.)

With this disclosure, a person having ordinary skill in this art **would not be lead to use this patent in formulating the oat-balancing feed supplement of the present invention.**

Reference No. 5

The Betz patent (a still further primary reference) is directed to the use of lemon oil in horse feeds. At column 1, line 64 – column 2, line 9, this patent discloses:

> In accordance with the present invention, lemon oil when added to all classifications of horse feeds will result in improved palatability. The feeds are generally indexed according to the

> percentage of total digestible nutrients such as carbohydrates, fats and proteins. Accordingly, the feeds of this invention will include, for example, concentrates which contain at least 65% total digestable nutrients but are low in fiber (less than 10%), and roughage which is low in total digestible nutrients but proportionately high in fiber. The concentrates may be carbonaceous concentrates which are low in protein but high in energy value or the concentrates may be protein supplements which as the name implies are higher in protein.

Thus, the Betz patent is directed to other issues regarding horse feeds and, therefore, fails to teach or suggest **the components of applicant's oat-balancing feed supplement in the quantities defined in applicant's amended claims**.

Reference No. 6

The Russian patent publication (a still further primary reference) is directed to a concentrated fodder and premix which can be mixed into coarse fodder for horse feed. However, this patent publication does not teach or suggest **the components of applicant's oat-balancing feed supplement in the quantities defined in applicant's amended claims**.

Reference No. 7

The Ott et al. reference (a still further primary reference) is directed to the growth and development effects of protein levels in the diet of a horse, especially yearling foals. However, as is true with the other six (6) primary references, this reference does not teach or

suggest **the components of applicant's oat-balancing feed supplement in the quantities defined in applicant's amended claims.**

Reference No. 8

The Graham reference (a secondary reference) is directed to the effects of lysine and threonine on the growth and development of yearling horses; refer to the Abstract on page 380. However, this reference adds nothing to the primary references in regard to the components of applicant's oat-balancing feed supplement **in the quantities defined in applicant's amended claims.**

Reference No. 9

Applicant acknowledges that the Lawrence reference (a tertiary reference) provides a guideline for feeding active horses in accordance with certain nutritional requirements. However, this reference (as is true with the secondary reference) **fails to provide the essential disclosure which is lacking in the other references** in regard to the components of applicant's oat-balancing feed supplement in the quantities defined in applicant's amended claims.

Reference No. 10

Applicant acknowledges that Horse Science reference (another tertiary reference) provides an overview of the daily nutritional requirements of horses. However, this reference also **fails to provide the essential disclosure which would lead one having ordinary skill in this art** to the components of applicant's oat-balancing feed supplement in the quantities defined in applicant's amended claims.

The above disclosures from the combination of multiple references cited by the Examiner under Section 103(a) show that these references do not recognize the problem faced by applicants and, therefore, do not disclose or suggest applicant's solution to such problem.

This rejection under Section 103(a) inherently leads to the conclusion that the primary references are viewed by the Examiner as not sufficient alone to support the rejection. Therefore, the questions are (1) whether the additional secondary and tertiary references provide the disclosure which is lacking in the primary references and (2) whether there is a valid basis to combine these references in the manner done by the Examiner.

In the Office Action, page 5, the Examiner states:

> "....it would have been obvious to one skilled in the art at the time the invention was made, to find such amounts based on each horse's needs by

As stated above, applicant is not claiming that the components of his oat-balancing feed supplement are new or have not been used in animal feeds. However, applicant does claim that the combination of oats and his oat-balancing feed supplement is new, unique, useful and not obvious from the cited referneces.

The Examiner's attention is directed to *KSR International Co. v. Teleflex Inc. et al.*, 127 S. Ct. 1727 (U.S. Supreme Court 2007), in which the Court states at 1741:

> When it first established the requirement of demonstrating a teaching, suggestion, or motivation to combine known elements in order to show that the combination is obvious, the Court of Customs and Patent Appeals captured a helpful insight. See *Application of Bergel*, 48 C.C.P.A. 1102, 292 F.2d 955, 956-957 (1961). As is clear from cases such as *Adams*, a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art. Although common sense directs one to look with care at a patent application that claims as innovation the combination of two known devices according to their established functions, it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does. This is so because inventions in most, if not all, instances rely upon building blocks long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known.

In view of the *KSR* decision, applicant submits that the Examiner's statements "the ingredients that are claimed in applicant's composition was well established at the time the invention was made, for feeding horses…." (pages 4-5 of the Office Action) and "….it would have been obvious to one skilled in the art at the time the invention was made, to find such amounts based on each horse's needs by using such worksheets, for each ingredient of the feed composition." (page 5 of the Office Action) are not valid bases for the combination of the primary references with the secondary and tertiary references. To constitute a valid basis, applicant submits that the Examiner must provide the reason by which a person of skill in this art (1) would "pick and choose" the various components **and quantities** from the secondary and tertiary references for addition to the primary references and (2) thereby produce an oat-balancing feed supplement as defined in the present application.

Based upon the above reasoning and amendments to the claims, applicant requests the removal of this rejection under Section 103(a).

Accordingly, applicant submits that this application is in condition for allowance, which action is requested.

Respectfully submitted,

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EQUINE GOITRE

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Summary

An investigation of equine goitre revealed a distinct association between the feeding of substances with a high iodine content to dams during pregnancy and the occurrence of this condition in their foals. Foetal deficiency of thyroid hormone was associated with stillbirths and the birth of weak foals.

Introduction

During the last century, a high incidence of goitre in man was observed in certain areas of Tipperary including Clonmel town (Hall, 1813; Molyneux, 1909). In his report of 1862, Benson stated that "goitre was quite common in and around Dublin". During the period 1946-1965, 1,065 cases of goitre in people of varying ages were treated in St. Vincent's Hospital, Dublin (Towers, 1966), one of a number of recognised treatment centres in the country. The incidence of the condition in Irish horses is unknown.

Materials and Methods

In the present investigation, three foals (Foals A, B and C) from a group of foals born to twelve mares on a stud farm during April-May, 1976, were studied. The foals had enlarged thyroid glands at birth; one of the foals was very weak at birth, but survived.

Serum thyroxine-binding globulin (TBG) levels in the affected animals were measured indirectly, on a *per cent* retention basis, by means of a simple I^{125} column system*. The test is based on the premise that there is a relatively constant amount of thyroid binding globulin in serum and that it binds most of the available thyroid hormone. A serum thyroxine-binding globulin content as represented on the basis of a percentage retention of 71.3 ± 2.7 was taken as the normal value for yearling thoroughbreds (Collins and Kelly, 1976).

The thyroid glands were measured with a calipers and their size was compared with that of normal glands. In the course of the investigation the three affected foals were treated with thyroid extract** at the rate of two mg. per kg. bodyweight daily, and their progress was monitored by evaluation of their serum T_3 levels.

During pregnancy the dams had been fed 5 lbs. of oats, 1 lb. of bran and 2½ lbs. of a

*Trilute System. Ames Company, Division Miles Laboratories Inc., Elkhart, Indiana.
**Eltroxin, Glaxo, Dublin.

Exhibit A

Table 1—Serum TBG Values of Three Goitrous Foals Prior To, During and Following Treatment with Thyroid Extract.

	Serum TBG % retention Pre-treatment	During Treatment	Post-treatment		
	May	June	July	Sept.	October
Foal A	62.1	70.9	69.4	70.9	71.3
Foal B	58.8	63.8	68.8	68.9	68.1
Foal C	45.9	57.1	67.9	63.0	61.1

propriatory conditioner daily. Analysis of pasture and soil from grazing paddocks was undertaken.

Data from the three clinically affected foals were related to autopsy findings in foals sent, for *post mortem* examination, to the Veterinary College during the period, 1965-1970.

Histological examination of the thyroid glands from the latter foals was carried out according to the methods of Dimoch, Westerfield and Doll (1944).

Results

When examined on May 25, 1976, all three foals showed enlarged thyroid glands, and the animals were backward in condition. The skeletal development of the foals was retarded. There was no evidence of goitre in the dams. Analysis of pasture and soil from grazing paddocks revealed normal levels of minerals and trace elements. On analysis, the supplementary conditioner was found to contain 20 *per cent* seaweed.

NB Each of the foals was treated with thyroid extract after which there was a noticeable improvement in their general physical condition. Serum TBG levels prior to, during, and following, treatment, are presented in Table (1).

Table (2) shows the diameter measurement (in mm) of the thyroid glands of the three foals in May, 1976 and April, 1977. Whilst the glands of Foal (A) showed considerable involution, thase of Foals (B) and (C) remained enlarged.

During 1976-1977 season the dams of the affected foals were kept under observation. Their diet was changed to include a supplement that did not contain seaweed or excess iodine. The serum TBG values of these mares, examined at monthly intervals, remained within the normal range. During the spring of 1977, each of the mares gave birth to a healthy foal that showed no evidence of thyroid enlargement.

Of 95 foals examined, *post mortem*, during 1965-1970, only one showed either gross or microscopic evidence of goitre. In the latter case, histological examination revealed glandular hyperplasia of the thyroid gland associated with incomplete follicle formation and epithelial stratification.

Table 2—Diameter of Thyroid Glands of Three Goitrous Foals During the First Months of Life and at One Year of Age.

		Diameter of thyroid gland May 1976	April 1977
Foal A	right gland	29 mm	16 mm
	left gland	24 mm	15 mm
Foal B	right gland	32 mm	30 mm
	left gland	29 mm	27 mm
Foal C	right gland	43 mm	24 mm
	left gland	30 mm	26 mm

Discussion

Peterson and Young (1952) demonstrated the transfer of thyroid hormone across the placental barrier. Nataf, Sfez, Michel and Roche (1956) reported that, following injection of radioactive triiodothyronine into pregnant rats, the concentration of this hormone in foetal serum reached the same level as that found in the dam's serum two hours after injection. More recently, Yamamoto, Onaya, Yamada and Kotani (1972) have shown that, under experimental conditions, excess iodine intake blocked or inhibited thyroid hormone secretion. On the other hand, Seidel, Klahn, Kuipel, Blodow, Heller and Loepelmann (1977) have shown that compensatory goitre in pigs is caused by iodine deficiency. In their investigation the latter workers found that up to 80 *per cent* of the iodine-deficient pigs had enlargement of the thyroid glands. Their clinical findings also demonstrated a significant reduction in weight gain. Lowe, Baldwin, Foote, Hillman and Kallfelz (1974) reported that horses affected with hypothyroidism also failed to gain in weight but responded well to thyroprotein supplementation. Excess dietary iodine intake by mares during pregnancy was identified by Drew, Barker and Williams (1975) as a major cause of goitrous conditions in the foal.

The findings reported here suggest that an association exists between the occurrence of goitre in foals and the feeding of excess quantities of iodine to their dams during pregnancy. The resulting parenchymatous goitre, which is attributed to excessive intake of iodine by the dams during pregnancy, may, in individual cases, lead to the birth of weak foals and stillbirths.

It is concluded also that in cases of parenchymatous goitre in foals, glandular involution following treatment is of long duration.

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We wish to thank William O'Carro[illegible] B.Agric.Sc., Agricultural Adviser, Co. Kildar[illegible] Committee of Agriculture, for carrying out th[illegible] soil and herbage analyses; Dr. Brendan [illegible] Farrelly, M.Sc., D.Vet.Med., M.R.C.V.S., De[illegible]partment of Veterinary Pathology and Micro[illegible]biology, U.C.D., for providing thyroid glanc[illegible] taken from foals presented, and Eamonn Fit[illegible]patrick, F.I.M.L.S., Chief Technician, Depar[illegible]ment of Veterinary Anatomy, U.C.D., for th[illegible] histological preparations.

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Analysis of Canadian and Irish forage, oats and commercially available equine concentrate feed for pathogenic fungi and mycotoxins

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Respiratory infections, recurrent airway obstruction (RAO) and exercise induced pulmonary haemorrhage (EIPH) are major causes of poor performance in horses. Fungi and mycotoxins are now recognised as a major cause of these conditions. The most notable fungi are *Aspergillus* and *Fusarium*. Fungal spores can originate from forage, bedding and feed and, in turn, these fungal spores can produce a series of mycotoxins as secondary metabolites.
This study set out to ascertain the degree of fungal and mycotoxin contamination in feed and fodder used in Irish racing yards over a one-year period. Weather conditions in forage producing areas were sampled by Met Eireann and the Canadian Meteorological Service.
Fifty per cent of Irish hay, 37% of haylage and 13% of Canadian hay contained pathogenic fungi. Of the mycotoxins, T2 and zearalenone were most prominent. Twenty-one per cent of Irish hay and 16% of pelleted feed contained zearalenone. Forty per cent of oats and 54% of pelleted feed contained T2 toxins.

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Key words: Aspergillus, mycotoxins, RAO, EIPH

Introduction

There are two main groups of fungi for concern in the equine world. They are the field fungus *Fusarium*, which produces the toxins fumonisin, zearalenone and T2, and the storage fungus *Aspergillus*, which produces the toxins aflatoxin and ochratoxin. *Aspergillus* is a toxigenic fungus which is ubiquitous in nature. Many species are identified, yet there are only three implicated in disease: *Aspergillus fumigatus*; *Aspergillus niger*; and *Aspergillus flavus*. The other important toxigenic fungus, in relation to disease in horses, is *Fusarium* because it is a potent producer of mycotoxins. Animals affected by mycotoxins may display symptoms such as digestive disorders, reduced feed consumption, poor thrift, impaired immunity, impaired reproduction and an undernourished appearance. They are not transmissible from animal to animal but are associated with consumption of infected feeds and forages (Quinn *et al.*, 2002). Several toxins have been linked with increased incidences of cancer in humans, notably aflatoxin, fumonisin, zearalenone and ochratoxin (Jacobsen *et al.*, 1999). Aflatoxin is a carcinogenic liver toxin which can suppress immunity and cause inappetance and ataxia. Fumonisins are carcinogenic to all animals and have long been associated with leucoencephalomalacia, a sporadic neurological disease in horses (Smith *et al.*, 1997). Ochratoxins and T2 toxin both interfere with protein synthesis and affect immune responses by suppressing antibody formation.

A performance horse is an athlete which cannot realise its full potential if its respiratory system is not functioning to the best of its ability. During peak performance, the racing Thoroughbred will inhale and exhale up to 15 litres of air in each of 150 breaths per minute, a total of over 2,000 litres per minute (Clarke, 2001). The ability of the airways to meet those massive demands can be compromised by inflammatory and/or allergic reactions induced by infectious agents, toxins, dust, or noxious gases. Recently, there has been growing recognition that some fungi, most notably *Aspergillus* and *Fusarium* species and their mycotoxins, exert seriously deleterious effects on the performance of the equine athlete (Clarke, 2001). Recurrent airway obstruction (RAO) is an allergic response which is comparable to asthma in humans. Fungal spores, which can originate from hay, bedding and feed, are the most common cause of RAO and can also cause exercise induced pulmonary haemorrhage (EIPH), also known as bleeding (Quinn *et al.*, 2002). Under certain conditions, fungal moulds grow unnoticed and can produce mycotoxins, toxic secondary metabolites. Mycotoxins are produced by fungi which can grow in baled hay, stored grain or silage with a high moisture content (Murphy, 1991) and it has been estimated that 25% of the world's crop production is contaminated with mycotoxins (Smith *et al.*, 1994; Smith *et al.*, 1997). These toxins can produce diseases known as mycotoxicosis and

Exhibit B

the symptoms of such diseases vary greatly. Mycotoxin effects are species specific and produce distinctly different symptoms in various animals (Osweiler, 1996). These can include digestive disorders, reduced feed consumption, general poor thrift, an undernourished appearance, immune suppression, subnormal production, impaired reproduction and/or a mixed, infectious disease profile (Whitlow and Haglar, 2002). In livestock, the five leading mycotoxins present in feed that impair growth and disrupt reproduction are aflatoxin, zearalenone, fumonisin, T2 and ochratoxin (Diekman *et al.*, 1992). In pigs, mycotoxins impair liver and kidney function and delay blood clotting and immune responses. Even limited exposure of *Fusarium* mycotoxins to pregnant swine can result in embryonic loss and disruption of normal reproductive cycling for an extended period of time (Long *et al.*, 1983). Mycotoxins may cause similar symptoms in horses as in other animals and, currently, research into the effects of mycotoxins in this species is ongoing. At present, literature points towards mould being one of the main factors in respiratory disease in horses both by ingestion and inhalation. In time, we hope to show, through research, that ingested mycotoxins may also play a negative role in the respiratory system.

In this study, samples of hay, haylage, oats and concentrate feed were taken at intervals over a one-year period and used to estimate the possible exposure of horses to moulds and their mycotoxins. Weather data for the two harvesting periods in which the feedstuffs were produced were examined to ascertain if there was an obvious relationship with the prevalence of mould in the samples.

Materials and methods

Sampling techniques

All of the samples tested were taken over a one-year period from October 1, 2002 to September 30, 2003, from equine racing yards in Leinster and Munster with horses in training. All of the Irish forage samples had been produced in the two provinces. The Canadian forage was produced in Calgary and Lethbridge.

All forage samples were taken from the centre of bales. Approximately 100g of fodder was collected by taking 20g aliquots from five different bales, from different areas of the fodder stack. The five aliquots were pooled and placed in a sterile plastic bag which was sealed. Sterile gloves were worn at all times and they were changed between samples.

All concentrate feed samples were taken from the centre of sealed bags. Approximately 100g of feed was collected by taking 20g aliquots from five different bags. The five aliquots were pooled and placed in a sterile plastic bag which was sealed. Sterile gloves were worn at all times and changed between samples.

All of the feed/fodder samples were ground using a centrifugal ultra mill (Retsch ZM100, Germany). The screen size was 1mm and the pre-selected speed 'K' was selected at 14,000 rpm. The appropriate grinding tool was inserted depending on the type of sample. A six-tooth rotor was used for fodders and nuts and a 12-tooth rotor was used for oats and coarse mixes. When the entire sample was loaded, the apparatus was allowed to run for one minute. The sample was then removed aseptically and placed in a 150ml container. The mill was sterilised between samples using Equisept disinfectant (Thoroughbred Remedies Ireland, Co.Kildare) at a concentration of 1400 ppm. The samples were stored at between 1°C and 4°C until extraction and analysis.

Sample extraction

As several mycotoxins were being tested for, several different extraction protocols were employed (Table 1).

<u>Extraction protocol 1</u>

This protocol was used on samples being analysed for aflatoxin-HS, total aflatoxin and zearalenone. The sample material was ground as fine as possible in a food blender. Five grammes of the sample material was added to a 100ml screw cap pot. Each pot was labelled with the unique NEOGEN sample code. Twenty-five millilitres of 70% methanol were added to each pot. The pot was shaken vigorously for three minutes. The sample material was allowed to settle for two to three minutes. The extract was poured into a sterile 1.5ml eppendorf until it was almost full. Each tube was labelled with the unique NEOGEN sample code, as allocated earlier. The tube was spun in the micro-centrifuge for three minutes at 10,000rpm. The sample extract was then ready for testing.

<u>Extraction protocol 2</u>

Protocol 2 was identical to protocol 1 except that 25ml of distilled water, instead of the 25ml of 70% methanol, was added.

<u>Extraction protocol 3</u>

Protocol 3 was identical to protocol 1 except for two changes: 10g, instead of 5g, of sample was used and 40ml of 50% methanol, instead of 25ml of 70% methanol, was added.

<u>Extraction protocol 4</u>

Protocol 4 was identical to protocol 1, except for one change: 25ml of 50% methanol, instead of 25ml of 70% methanol, was added.

Table 1: Extraction protocols used

Sample type	Aflatoxin	Ochratoxin	Vomitoxin	Zearalenone	T2	Fumonisin
Oats	1	3	2	1	4	5
Hay/silage	1	3	2	1	4	5
Coarse mix	1	3	2	1	4	5
Cubes	1	3	2	1	4	5

Table 2: Forage harvest climate statistics during 2002 and a 30-year period (1961-1990) for Leinster and Munster obtained from Met Eireann and Canadian Meteorological Service

	May						June						July					
	Rainfall (mm)		Mean temp. (°C)		Rel. humidity (%)		Rainfall (mm)		Mean temp. (°C)		Rel.humidity (%)		Rainfall (mm)		Mean temp (°C)		Rel.humidity (%)	
Location	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean
Birr	109.6	61.7	15.8	14.9	68.0	64.0	96.2	55.2	18.8	17.7	71.0	66.0	68.2	59.1	20.1	19.2	69.0	67.0
Dublin	121.3	55.1	14.1	14.2	72.0	67.0	81.2	56.0	17.4	17.2	73.0	68.0	68.9	49.9	19.4	18.9	71.0	68.0
Kilkenny	105.4	61.9	14.8	15.1	69.0	64.0	80.4	50.5	17.7	18.1	69.0	65.0	74.4	52.5	19.4	19.9	69.0	65.0
Mullingar	117.6	72.4	15.2	14.7	68.0	68.0	89.6	66.2	17.1	17.5	81.0	79.0	81.3	61.8	18.9	19.0	74.0	70.0
Rosslare	89.1	55.5	14.1	13.2	79.0	77.0	49.8	47.6	16.4	15.9	82.0	78.0	36.0	50.7	18.1	17.9	76.0	77.0
Cork	162.8	83.4	14.8	13.8	74.0	71.0	105.2	68.8	17.7	16.6	77.0	72.0	54.0	66.4	17.7	18.5	76.0	72.0
Shannon	139.6	60.1	15.9	15.3	68.0	64.0	89.8	62.4	18.2	17.9	71.0	67.0	68.1	57.1	18.9	19.4	71.0	68.0
MEAN	120.8	64.3	14.9	14.5	71.1	67.9	84.6	58.1	17.6	17.3	75.0	71.0	64.4	56.8	18.9	19.0	72.3	69.6
Calgary	10.9	51.4	12.9	16.4	40.6	42.8	58.6	79.8	21.3	20.2	46.0	45.8	34.6	67.9	26.1	22.9	42.4	45.7
Lethbridge	31.5	49.4	15.1	18.2	39.8	40.3	25.1	63.0	21.5	22.3	40.8	41.4	34.0	47.5	27.3	25.5	40.6	39.9
MEAN	21.2	50.4	14.0	17.3	40.2	41.7	41.9	71.3	21.4	21.3	43.4	43.6	34.3	57.7	26.7	24.2	41.5	42.8

Extraction protocol 5

Protocol 5 was identical to protocol 1 except for one change: 100 µl of the sample was extracted to a sample dilution bottle and mixed as per manufacturer's per manufacturer's (Neogen Corporation, Nellies Gate, Ayr, Scotland) instructions.

ELISA procedure

ELISA testing was carried out as per manufacturer's (Neogen Corporation, Nellies Gate, Ayr, Scotland) instructions.

Culture techniques and identification

Ten grammes of each sample was added to 90ml of sterile distilled water in a labelled 150ml container. The containers were then placed on a shaker for ten minutes. One millilitre of each of the samples was then pipetted onto a correspondingly labelled Sabouraud dextrose agar plate (Oxoid, Basingstoke). Using a spreader, the solution was spread evenly over the plates. The plates were then incubated at 25°C for five days. After incubation, all plates were examined for fungal growth using conventional methods. The cultures were examined using the sellotape method. A loop was made with a length of sellotape. The base of the loop was touched gently onto the fungal culture so that some of the mycelium adhered to the tape. A drop of lactophenol cotton blue was placed on a microscope slide. The sellotape was placed on top of the stain and pressed down lightly. The slide was then examined under X10 and X40 magnification. The different species of fungi were characterised by colonial morphology and microscopy.

On culture, typical colonies were counted and noted. The cultures are a result of an earlier 1/10 dilution. It was decided to group the growths of fungi into light, moderate and heavy growths:

Light growth = 1-6 typical colonies = 10–60 cfu/g of sample
Medium growth = 7-12 typical colonies = 70–120 cfu/g of sample
Heavy growth = over 12 typical colonies = >120 cfu/g of sample
cfu: colony forming unit

Table 3: Cereal harvest climate statistics during 2002 and a 30-year period (1961-1990) for Leinster and Munster obtained from Met Eireann and Canadian Meteorological Service

	August						September					
	Rainfall (mm)		Mean temp (°C)		Rel.humidity (%)		Rainfall (mm)		Mean temp (°C)		Rel.humidity (%)	
Location	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean	2002	30 Yr Mean
Birr	44.2	77.6	18.0	18.8	68.0	68.0	22.6	70.6	16.0	16.6	71.0	72.0
Dublin	50.8	70.5	18.1	18.6	71.0	70.0	22.6	66.7	16.9	16.6	69.0	70.0
Kilkenny	52.9	69.4	19.4	19.6	64.0	66.0	19.7	73.5	17.4	17.2	68.0	69.0
Mullingar	62.9	81.2	18.6	18.6	60.0	72.0	18.9	85.9	16.7	16.4	71.0	74.0
Rosslare	40.7	68.7	17.7	17.9	75.0	78.0	12.4	73.3	16.4	16.3	76.0	76.0
Cork	47.5	88.7	18.0	18.2	73.0	73.0	35.6	96.4	16.4	16.0	72.0	76.0
Shannon	48.1	82.3	18.7	19.2	67.0	69.0	19.4	81.8	17.5	17.2	68.0	71.0
MEAN	49.6	76.9	18.4	18.7	70.0	71.0	21.6	78.3	16.8	16.6	71.0	73.0
Calgary	57.4	58.7	21.1	22.5	44.6	44.8	54.0	41.7	16.4	17.6	45.0	45.1
Lethbridge	42.4	45.1	22.0	25.4	56.5	56.8	48.3	37.6	18.8	20.1	52.9	53.1
MEAN	49.9	51.9	21.5	24.0	51.0	50.8	51.0	39.7	17.6	18.0	49.0	49.1

Table 4: Pathogenic fungi isolated from forage/feed during the period October 1, 2002 and September 30, 2003.

Fodder type	Number sampled	Pathogenic fungi isolated No.	%	Number A. fumigatus	Number A. niger	Number A. flavus	Number Fusarium
Canadian hay	63	8	13	4	4	2	0
Irish hay	62	31	50	9	11	11	0
Haylage	54	20	37	16	2	2	0
Coarse mix	38	5	13	0	2	2	1
Oats	26	2	8	0	1	1	0
Pelleted feed	51	2	4	0	0	2	0

No. = number positive % = % positive

Meteorological data

Harvesting of forage, in the northern hemisphere, occurs predominantly in the months of May to July. Harvesting of grain crops occurs during the months of August to September. Five to seven days of good dry weather is required for haymakers. Haylage requires three to four days whereas grain harvest requires dry conditions at harvest time.

Monthly data reports of total rainfall, relative humidity and temperature for those months in 2002 were obtained from the Met Eireann and the Canadian Meteorological Service stations of the regions which the forage and crops were produced. Weather and crop reports were also obtained from Teagasc, the Irish farm advisory service, for haymaking.

Results

Tables 2 to 5 show the results obtained in the study. Note that any species of non-pathogenic fungi that were isolated (e.g., *Mucor* and most *Penicillium* sp) were not included in the results.

For each of the three months of the forage harvest period (May, June and July of 2002), data from seven weather stations in Leinster/Munster and two from Canadian areas, where the sampled forage was produced, were used to derive mean weather statistics (Table 2). The most striking observation is the increase in rainfall and relative humidity in 2002 compared to the 30-year mean for the Irish weather stations. During the same period in Canada, there was an overall reduction.

The results of Table 3 show the climatic conditions from seven meteorological stations in Ireland and the two Canadian stations in August and September, detailing the rainfall, temperature and the relative humidity.

Table 4 shows the number of samples tested during the period of October 1, 2002 to September 30, 2003, including those that tested postive for pathogenic fungi. A greater percentage of Irish hay and haylage samples tested positive than the Canadian hay samples. The amount of pathogenic fungi isolated from the feed samples, i.e., oats, coarse mix and pellets, was significantly lower than in fodder. *Aspergillus fumigatus* was isolated on 16 occasions from haylage. *Fusarium*, the field fungus, was not isolated from any of the fodder samples and only once in a feed sample.

Table 5 shows the distribution of five mycotoxins in feed and forage: the number sampled, the number above the recommended level; and, the percentage above the recommended levels. The recommended levels are based on FDA (US Food and Drug Administration) and EU advisory levels for mycotoxins in horses and are as follows:

- Aflatoxin – 50 ppb (legislation)
- Zearalenone – 150 ppb (recommended)
- Fumonisin – 50 ppm (recommended by FDA)
- Ochratoxin – 20 ppb (recommended)
- T2 toxin – 150 ppb (recommended)

Aflatoxin, as seen from the data, was not an issue in the tested season. The same cannot be said for ochratoxin and fumonisin, except for the coarse mix samples which had a small number of positives. All of the feed types, apart from oats, contained positives for zearalenone with the Irish hay being the most significant. None of the forage samples contained T2 toxins but 14% of the coarse mix, 40% of the

Table 5: Mycotoxin results for forage and concentrates

Fodder type	Zearalenone No.	%	Ochratoxin No.	%	Aflatoxin No.	%	T2 Toxin No.	P (%)	Fumonisin No.	%
Irish hay	44	9 (21)	15	0 (0)	44	0 (0)	15	0 (0)	15	0 (0)
Haylage	40	3 (8)	34	0 (0)	40	0 (0)	34	0 (0)	34	0 (0)
Canadian hay	65	5 (8)	0	0 (0)	65	0 (0)	0	0 (0)	0	0 (0)
Coarse mix	35	2 (6)	28	1 (4)	35	0 (0)	28	4 (14)	31	2 (6)
Oats	25	0 (0)	16	0 (0)	24	0 (0)	17	7 (40)	17	0 (0)
Pelleted feed	50	8 (16)	50	0 (0)	50	0 (0)	50	27 (54)	50	0 (0)

No. = number positive % = % positive

oats and 50% of pelleted feed samples tested contained T2 toxins above the advisory or recommended levels.

Discussion

Fungal spores are the most common cause of RAO (Clarke, 2001). They can also contribute to EIPH, also known as 'bleeding'. Spores are highly antigenic and cause much damage to humans and animals if inhaled or ingested. Fungi also produce mycotoxins which are toxic secondary metabolites. They are produced during the secondary sporulation stage in response to a stressful situation and so are like a defence mechanism for the fungi (Clarke, 2001). Zearalenone is an oestrogenic toxin that can lead to reproductive problems and also cause anorexia, diarrhoea and dehydration (Smith *et al.*, 1997).

The fungal spores which affect horses predominantly originate from feed and fodder. It has been estimated that 25% of the world's crop production is contaminated with mycotoxins (European Commission Services, 1994). Ireland's temperate climate can often present difficulties when it comes to providing suitable feed and fodder for horses. Problems arise during the production of hay as dry weather is required to reduce moisture content to 15-20%. Haylage, however, only needs two days to wilt the grass before it is wrapped to preserve. For cereals, the weather at harvest time needs to be dry in order to reduce the moisture content to sufficiently inhibit mould growth. Toxigenic mould spores can proliferate when hay is baled damp, as can happen during a summer with a large volume of rain. If hay heats, *Aspergillus fumigatus* can often be found. Haylage may require less suitable weather but can also have problems. Producers often attempt to make hay, fail and then wrap it and call it haylage. This is unsuitable as little respiration will take place, aerobic conditions may exist and moulds will grow. Once haylage is produced, care has to be taken not to puncture the plastic that would allow both moisture and air to enter, which will encourage fungal growth. Unlike hay, once haylage is opened it has to be used within seventy-two hours or, again, fungi will proliferate.

It has been identified that wet, humid weather at flowering promotes infection by *Fusarium* of grain and grasses (Whitlow and Hagler, 2002). *Fusarium* is a field fungus and it has been shown that, whilst growing on grain, it can be inhibited and overgrown by *Aspergillus* during storage (Smith *et al.*, 1997). Storage of feed and fodder is critical. High moisture and relative humidity can lead to an explosion of mould growth. Concentrated feeds are predominantly made from grains, they have their advantages but each ingredient is capable of contributing to the overall fungal and mycotoxin load. The increasing price of soya has led to some companies using citrus pulp and pine kernels instead, which may account for increased mycotoxin production. The varying stresses of heating and drying during processing of concentrates may encourage fungal damage and mycotoxin release. The competition for nutrients between fungi from harvest to storage may result in enough stresses for one or both to produce mycotoxins. There may also be a synergistic effect whereby products and toxins produced by one fungi may aid the propagation of another.

However, moisture is the undeniably important factor when discussing quality feed production for horses. Dry weather at harvest is critical in attempts to limit fungal growth and subsequent mycotoxin production. In 2002, the mean rainfall for the five weather stations examined during the fodder harvest period was well in excess of the mean for the previous thirty years for those stations. In fact, the mean rainfall in the month of May was almost double the 30-year mean for that month. During the grain harvest, the rainfall figures dropped dramatically to two-thirds of the yearly mean in August and then to a quarter of the yearly mean in September. On collating these facts with the literature, a fodder crop with high fungal activity and a grain crop with less fungal activity can be predicted. The variance between the two hays and the haylage were quite significant. Of the sixty-three Canadian hays sampled, only eight (13%) contained pathogenic fungi. Of the eight positives, 50% were *A. fumigatus* with the remaining fifty divided between *A. niger* and *A. flavus*. No *Fusarium* was isolated. The Irish hay did not fare as well. Thirty-one (50%) of the sixty-two Irish hays sampled contained pathogenic fungi. No *Fusarium* was isolated: all were *Aspergillus*, including *A. fumigatus*, *A. niger* and *A. flavus*. The haylage proved superior to the Irish hay but not as good as the Canadian hay. Twenty of the fifty-four sampled contained pathogenic fungi with *Aspergillus fumigatus* accounting for 80% of the positives. Again, no *Fusarium* was isolated. A number of points arise from this data. Firstly, the excessive rainfall during the fodder harvest in Ireland has contributed to the production of hay of which 50% contains pathogenic fungi. Compare this to the Canadian hay which contained 13% positives – this hay was produced in a much drier climate which has an overall 25-30% lower humidity during forage harvest periods as compared to Ireland. No *Fusarium* was isolated from forage which would suggest either none existed pre-harvest, or that *Aspergillus* propagated during storage.
The haylage showed superior results than the Irish hay at 37% positive, but it was still a very high figure. Of the positives, 80% contained *Aspergillus fumigatus* which complements the characteristic that this particular strain can survive oxygen depletion.

The results of the concentrated feed fungal analysis present a much different picture. Thirty-eight coarse mixes were sampled, of which five (13%) contained pathogenic fungi. Two contained *A. niger*, two *A. flavus* and one sample contained *Fusarium*. Twenty-six oat samples were tested with only two or 8% containing pathogenic fungi, 50% being *A. niger* and 50% being *A. flavus*. Of the fifty-one pelleted feed samples, only two, or 4%, contained pathogenic fungi - both *Aspergillus flavus*. More interestingly, the below-average rainfall seems to have contributed to a low fungal load as compared to the forage harvest. The coarse mix contained the highest percentage. The oats were next, with the pelleted feed containing the least

amount of pathogenic fungi. This would suggest that the heat treatment pelleted feed undergoes decreased the fungal load. No *Aspergillus fumigatus* was isolated but one coarse mix contained *Fusarium*. The weather at the time of harvest, coupled with good storage conditions, have contributed to concentrated feed in 2002 with a relatively low fungal load.

The literature states that zearalenone is produced by *Fusaria* spp. Only one sample contained *Fusarium* yet all the different types of feed and forage except oats contained zearalenone above the recommended level (Canadian hay 8%, Irish hay 21%, haylage 8%, coarse mix 6% and pelleted feed 16%). The Irish hay again was the worst in this regard. Five of the nine positives also contained *Aspergillus* species. This would suggest that *Fusarium* existed in both crops and forage pre-harvest, died off and produced zearalenone in doing so, with *Aspergillus* taking over as a storage fungi in some cases. Osweiller (1996) described zearalenone concentration in US hay as rarely, if ever, occurring. This is not the case in Ireland where 21% contained zearalenone above the recommended levels. None of the oats contained zearalenone, which suggests some of the other ingredients involved in production of the pelleted feed and coarse mix were responsible for the zearalenone contamination.

Fumonisin is also produced by *Fusarium* yet no samples tested contained this toxin, except in the case of 6% of the coarse mixes. Again, as the oats were negative, one of the other ingredients is responsible for the contamination. None of the feedstuffs tested positive for ochratoxin except for coarse mix, where 6% of the samples contained this toxin above the recommended level. Ochratoxin is produced by the toxigenic strains of *Aspergillu ochraceus* and *Penicillium viridicatum*, neither of which were isolated during the study. Aflatoxin is produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The latter was not isolated during this study. *Aspergillus flavus*, however, was isolated on seventeen occasions throughout the study yet none of those samples contained aflatoxin above the legal/recommended levels. This would suggest that the production of the feeds and fodders did not stress the fungi sufficiently to release the toxin, nor did the fungi reach sporulation stage. T2 toxin is a trichothecene produced by *Fusarium*. It was not detected in any of the fodder samples tested. However, 14% of the coarse mix, 40% of the oats and 54% of the pelleted feeds tested contained T2 toxin above the recommended levels. This suggests that *Fusarium* was present pre-harvest and a combination of dry weather and production processes removed the fungi, but the fungi produced significant amounts of toxin which remained stable during production and storage. Another theory to add to this may be that the very moist weather early in the summer may have increased *Fusarium* growth. The dry spell which followed this may have aided the demise of the fungi resulting in a mass of toxin being produced.

Conclusions

Moulds and mycotoxins are a cause of health problems in horses and other animal species. The results of our paper show that levels of pathogenic fungi and mycotoxins are present in animal feedstuffs. Legislation is currently being drafted at EU level to address the problem of mycotoxins in animal feeds. To date, legislation only covers aflatoxins. The new legislation will cover a range of mycotoxins with acceptable levels that will need to be adhered to. Feed companies will need to have a mycotoxin screening programme on all the ingredients that make up their products. Shelf-life studies may also need to be put in place to ensure that no deterioration in feed occurs.

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